1 of 4

NASA TECHNICAL TRANSLATION NASA TT F-15,647

PRESENCE AND REGULATION OF THE SYNTHESIS OF TWO ALCOHOL DEHYDROGENASES FROM SACCHAROMYCES CEREVISIAE

L. Schimpfessel

(NASA-TT-F-15647) PRESENCE AND REGULATION OF THE SYNTHESIS OF TWO ALCOHOL DEHYDROGENASES FROM SACCHAROMYCES CEREVISIAE (Kanner (Leo) Associates) 21 p HC $4.25 CSCL 07C G3/06

N74-22801

Unclas 38969

Translation of "Presence et regulation de la synthese de deux alcool deshydrogenases chez la levure Saccharomyces cerevisiae," Biochimica et biophysica acta, Vol. 151, 1968, pp. 317-329

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
WASHINGTON, D.C. 20546 MAY 1974

STANDARD TITLE PAGE

1. Report No. NASA TT F-15,647	2. Government Accession No.	3. Recipient's Catalog No.
4. Title and Subtitle PRESENCE AND REGULATION OF THE SYNTHESIS OF TWO ALCOHOL DEHYDROGENASES FROM SACCHAROMYCES CEREVISIAE		5. Report Date May 1974 6. Performing Organization Code
7. Author(s) L. Schimpfessel, Research Institute, Learning & Research Center of the Food and Chemical Industries, Brussels.		8. Performing Organization Report No. 10. Work Unit No.
9. Performing Organization Name and Address Leo Kanner Associates, P.O. Box 5187, Redwood City, California 94063		11. Contract or Grant No. NASw-2481 13. Type of Report and Period Covered Translation
12. Sponsoring Agency Name and Address NATIONAL AERONAUTICS AND SPACE ADMINISTRATION, WASHINGTON, D.C. 20546		14. Sponsoring Agency Code

15. Supplementary Notes

Translation of "Presence et regulation de la synthese de deux alcool deshydrogenases chez la levure Saccharomyces cerevisiae," Biochimica et biophysica acta, Vol. 151, 1968, pp. 317-329.

16. Abstract (1) Saccharomyces cerevisiae is able to synthesize two different alcohol dehydrogenases (alcohol: NAD oxidoreductase, EC 1.1.1.1) according to the culture carbon substrate: the alcohol dehydrogenase I produced by the yeast grown on glucose (aerobic or anaerobic growth) is the fermentative alcohol dehydrogenase, and the alcohol dehydrogenase II produced by the yeast growth on lactate or ethanol as a sole source of carbon and energy, is the oxidative alcohol dehydrogenase. (2) The difference between the two enzymes is shown by the study: (a) of their thermostability, (b) of their substrate specificity. (3) These two enzymes have common properties: they require NAD as cofactor and have the same optimal pH. (4) The alcohol dehydrogenase II is repressed by glucose.

17. Key Words (Selected by Author(s))	18. Distribution Statement
	UNLIMITED

19. Security Classif. (of this report)	20. Security Classif. (of this page)	21. No. of Pages	22. Price
Unclassified	Unclassified	21	4.25

NASA-HQ

PRESENCE AND REGULATION OF THE SYNTHESIS OF TWO ALCOHOL DEHYDROGENASES FROM SACCHAROMYCES CEREVISIAE

L. Schimpfessel*

Introduction

/317**

The Saccharomyces cerevisiae yeast can grow using ethanol as the only source of carbon, regardless of whether the ethanol is provided as the sole source of carbon, or whether after an aerobic growth on glucose, the yeast assimilates the ethanol, a product of the aerobic fermentation, accumulated in the medium. The curves of prolonged growth on glucose reveal this phenomenon by the appearance of a double growth.

This work is devoted to the study of the first enzyme of ethanol assimilation in S. cerevisiae: the alcohol dehydrogenase (alcohol: NAD oxidoreductase, EC 1.1.1.1). Actually, the growth of the yeast on ethanol involves the oxidation of alcohol in acetate with the transient formation of acetaldehyde. The presence of enzymes involved in this oxidation -- the alcohol dehydrogenase /318 that oxidizes the ethanol to acetaldehyde and the aldehyde dehydrogenase that oxidizes the acetaldehyde to acetate -- has been duly established in the case of S. cerevisiae.

While the alcohol dehydrogenase of yeast has been the object of a number of studies both from the standpoint of its structure and from the standpoint of its working mechanism, few works, however, have dealt with its regulation. Since it is only slightly subject to substantial fluctuations, this enzyme was deemed "constitutive"

---

* Research Institute, Learning and Research Center of the Food and Chemical Industries (C.E.R.I.A.), Brussels, and of the Laboratory of Microbiology of the University of Brussels, Brussels, Belgium.

**Numbers in the margin indicate pagination in the foreign text.

in yeast until lately.

But in 1957, Galzy and Slonimski [3] observed higher rates of alcohol dehydrogenase activity in S. Cerevisiae grown in a medium where lactate is the sole source of carbon than in yeast grown on glucose. The significance of these high alcohol dehydrogenase contents on lactate did not, however, appear immediately, even considering the possible dual role of the reaction catalyzed by the alcohol dehydrogenase. This observation induced us to resume the study of alcohol dehydrogenase regulation in yeast. The details of our results are presented hereinbelow; a brief summary of these results has already been published [8, 10]. There are two independently regulated alcohol dehydrogenases (I and II), and the catabolic derepression of dehydrogenase II is responsible for the sharp variations observed. More recently, Witt, Kronau and Holzer [11], on the one hand, and Hommes [4], on the other, confirmed the variations of the overall alcohol dehydrogenase content without making a distinction between the two forms of alcohol dehydrogenase. These authors showed that the alcohol dehydrogenase activity rate can vary considerably in yeast, depending on whether it has glucose or not, and when it does, depending on the initial glucose concentration in the medium. Holzer deduced that the alcohol dehydrogenase synthesis is repressed by glucose. This glucose effect at the alcohol dehydrogenase level permits control of the intensity of gluconeogenesis from ethanol, which becomes useless when the cell has glucose for its growth.

In the case of S. cerevisiae, this work establishes the individuality of two alcohol dehydrogenases involved in different metabolisms. In 1957, Ebisuzaki and Barron [2] had already described the presence of a second alcohol dehydrogenase in yeast without attributing a specific role to this new enzyme in its metabolism. Actually, while the classic alcohol dehydrogenase of yeast (alcohol dehydrogenase I) is directly linked to glycolysis

where it ensures the reoxidation of NADH while reducing the acetaldehyde to ethanol, the new alcohol dehydrogenase (alcohol dehydrogenase II) ensures the entry of ethanol in the metabolism by oxidizing it to acetaldehyde. The synthesis of this latter alcohol dehydrogenase is the one that is sensitive to repression by glucose. The two enzymes have been distinguished on the basis of their enzymatic specificity, their thermostability and their heat inactivation kinetics.

Material and Methods

Yeast Strain

The yeast strain used is a pure strain of baker's yeast (Saccharomyces cerevisiae), normal diploid "yeast foam."

Culture Media

The complex natural medium with glucose contains, per liter: yeast extract, 2.5 g; KH_2PO_4, 2.7 g; $(NH_4)_2SO_4$, 2.6 g; $MgSO_4 \cdot 7H_2O$, /319 0.25 g; glucose, 20.0 g. The solution is carried to a final pH of 6.5 and sterilized in an autoclave.

The culture medium with ethanol is the same complex natural medium as the preceding one, but without glucose. The solution is carried to a final pH of 5.0 and sterilized in an autoclave. 20 ml of ethanol sterilized by filtration are added per liter of medium in a sterile manner.

The culture media with acetate and lactate are prepared substituting for glucose either 6 g per liter of potassium acetate, or 22.4 ml per liter of a 50% solution of sodium lactate. The solutions are carried to a final pH of 5.0 and sterilized.

Growth of Yeasts

Growths, followed with a Beckman B spectrophotometer at 660 nm, are carried out at 30°. Aeration of the cultures is provided by constant stirring of the balloons on the mechanical incubator.

Anaerobic cultures are produced in 6-liter balloons containing 4 liters of medium stirred by means of a magnetic bar in a nitrogen atmosphere. The cells are collected by centrifugation in the exponential phase of growth (degree of absorption 1.2).

Preparation of the Extracts

After centrifugation of the culture at 0° for 4 min at 3000 x *g*, the yeast deposits are washed in distilled water and returned to suspension in a 0.05 M phosphate buffer (pH 7.7) at the rate of about 5 g of moist weight and 15 ml of the buffer. This suspension is then exposed to supersonic vibrations for 5 min in a Raytheon apparatus (10 Kc - 250 W). The total extract obtained is then centrifuged at 3000 x *g* for 5 min: the deposit obtained consists of two layers: a lower layer, consisting of intact cells; the other one, an upper layer, consisting of cell walls. The supernatant, which consists of the raw extract is maintained at 0°. Finally, the raw extract is centrifuged at 100,000 x *g* for 30 min, resulting, on the one hand, in a gelatin-like deposit consisting of the cytoplasmatic granulations and, on the other hand, the supernatant liquid or soluble fraction.

The proteins are determined by the biuret method.

Measurement of the Alcohol Dehydrogenase Activity

The alcohol dehydrogenase is determined in the soluble extract according to Racker's [7] method, with respect to oxidation of ethanol and acetaldehyde.

In the presence of an excess of ethanol the reduction rate of NAD is proportional to the enzyme concentration. The formation of NADH is followed in the Beckman DK_1 with an optical path of 1 cm and a wavelength of 340 nm.

The specific activities of alcohol dehydrogenase are expressed in rational units, i.e., in μM of NADH formed by h/mg proteins, bearing in mind the coefficient of molar extinction of NADH ($6.22 \cdot 10^3$).

Results

/320

Development of the Alcohol Dehydrogenase Activity During the Growth of S. Cerevisiae on Glucose

A prolonged growth of S. cerevisiae on glucose involves, essentially, three distinct phases: (1) the exponential phase of growth on glucose where the yeast ferments the glucose producing ethanol, which accumulates in the medium; (2) the phase of slackening and latency where the exhaustion of glucose in the medium causes the arrest of growth at the expense of this sugar; and (3) the phase of exponential growth on ethanol where the yeast assimilates the ethanol accumulated during fermentation (see Fig. 1).

In order to establish a possible correlation between the evolution of the cell physiology during this double growth, and the enzyme equipment of the yeast, determinations of the alcohol dehydrogenase activity were conducted with the soluble fractions of yeast extracts collected throughout growth.

A noticeable increase in the specific activity of this enzyme occurs during phases (2) and (3) (see Fig. 1) at the time when the yeast, having exhausted the glucose present in the medium, adjusts to reduce the accumulated alcohol.

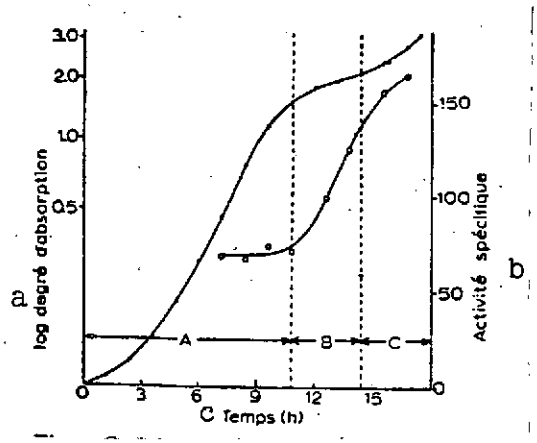

Fig. 1. Growth of S. cerevisiae on glucose (●) and specific activities of alcohol dehydrogenase (o). For details of the phases, see text.

Key: a. Log degree of absorption
b. Specific activity
c. Time (in hours)

Culture Carbon Source and Rate of Activity of Alcohol Dehydrogenase

The determination of the rates of activity of alcohol dehydrogenase in the raw extracts of S. cerevisiae after growth on different carbon substrates has yielded the following results (see Table I).

All of the yeast extracts grown on ethanol, lactate, or glycerol as the sole source of carbon exhibit much higher alcohol dehydrogenase contents than the yeasts cultivated on glucose.

TABLE I. RATE OF ACTIVITY OF ALCOHOL DEHYDROGENASE ACCORDING TO THE NATURE OF THE CULTURE CARBON SUBSTRATE.

Carbon Source	Duration (g) of Growth (min)	Specific Activity (units) of Alcohol Dehydrogenase of Yeasts Collected in the:	
		Exponential Phase	Stationary Phase
Glucose	45	20	60
Ethanol	260	170	250
Lactate	260	200	260
Acétate	160	110	—
Glycérol	260	250	—

Comparative Study of Alcohol Dehydrogenase Activities in S. Cerevisiae According to the Nature of the Culture Carbon Substrate

Thermostability. In order to determine the respective thermostability of the different alcohol dehydrogenase preparations, /321 soluble extracts are exposed for 5 min to the action of increasing temperatures, and their residual activity is determined at the regular temperature (Fig. 2). The alcohol dehydrogenase of the extracts from yeasts cultivated on ethanol (extract II) exhibit a greater thermostability than the thermostability of the aerobic or anaerobic yeasts on glucose (extract I).

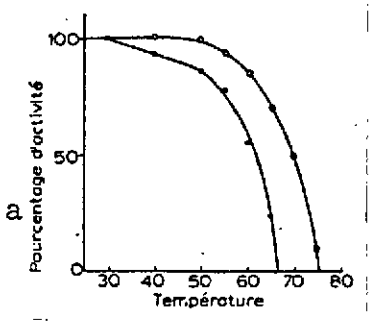

Fig. 2. Residual activity of alcohol dehydrogenase after a 5-min heat treatment at different temperatures: ●, yeast extract cultivated on glucose; o, yeast extracts cultivated on ethanol.

Key: a. Percentage of activity

Insofar as the extract on lactate is concerned, it might consist of a mixture of the two types of alcohol dehydrogenase.

The inactivation temperature, or the temperature at which a 5-min treatment suffices to in- /322 activate 50% of the initial activity of alcohol dehydrogenase (according to Kaplan [5]) is derived from these measurements of thermostability (Table II).

TABLE II. TEMPERATURE OF INACTIVATION OF ALCOHOL DEHYDROGENASE ACCORDING TO THE ORIGIN OF THE EXTRACT.

Origin of Extract	Inactivation Temperature	Type of Alcohol Dehydrogenase
Glucose/air	59°	Alcohol dehydrogenase I
Glucose/N_2	60°	Alcohol dehydrogenase I
Ethanol	70°	Alcohol dehydrogenase II
Lactate	66.5°	Alcohol dehydrogenase I and II

Heat Inactivation Kinetics

For the purpose of quantitatively evaluating the relative heat resistance of alcohol dehydrogenases, soluble extracts of yeast on glucose (I) and on ethanol (II), previously diluted so as to include the same protein concentrations, as well as a mixture of the two extracts, are exposed to the action of heat, at specific temperatures (53°, 57°, and 61°) for different intervals; their residual activity is then measured at room temperature [12].

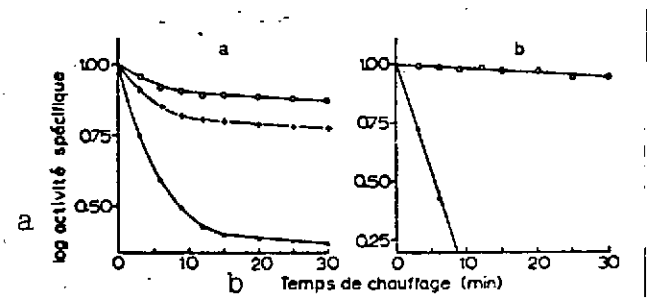

Fig. 3. Heat denaturation of alcohol dehydrogenases at 61°. a. Heat inactivation kinetics of alcohol dehydrogenase I (•), of alcohol dehydrogenase II (o) and of a mixture (+). b. Inactivation lines of alcohol dehydrogenase I (•) and II (o) calculated on the basis of the data of a.

Key: a. Log of specific activity
b. Heating time (min)

Figure 3a represents the inactivation kinetics at 61°. Thermodenaturation leads, both for the extracts treated separately and for the mixture, to complex kinetics which reveal the heterogeneity of the three samples. But while the alcohol dehydrogenase present in extract I (glucose) is inactivated by more than 90% after 12 min at 61°, the enzyme of extract II (ethanol) retains almost 90% of its initial activity after this treatment. This confirms that the alcohol dehydrogenases present in the two types of extracts differ at least from the standpoint of their thermostability. The inactivation lines of the two types of alcohol dehydrogenases were calculated on the basis of the experimental data (Fig. 3b) and permitted the determination of the respective half-life of the two components (Table III).

TABLE III. HALF-LIFE OF ALCOHOL DEHYDROGENASES AT 61°.

Alcohol Dehydrogenase Type	Half-Life at 61°
Alcohol dehydrogenase I	1 min 39 sec
Alcohol dehydrogenase II	87 min 45 sec

These values are encountered again both for the components of the extract on glucose and for those of the extract on ethanol, thus confirming the identity of the two components that constitute the extract in both cases.

The extrapolation of zero time of the linear portions of the /323 two curves makes it possible to determine the percentage of thermosensitive (alcohol dehydrogenase I) and heat-resistant (alcohol dehydrogenase II) components in the different extracts (Table IV).

TABLE IV. ALCOHOL DEHYDROGENASE I AND II CONTENT OF THE DIFFERENT EXTRACTS.

Origin of the Extract	Alcohol Dehydrogenase I (%)	Alcohol Dehydrogenase II (%)
Glucose/air (exponential phase)	93	7
Glucose/air (stationary phase)	64	36
Glucose/nitrogen	98	2
Ethanol	37	63
Lactate	55	45

Enzymatic Specificity

The comparison of the oxidation rate of different alcohols, under standard conditions, by the soluble fractions of yeast extracts has made it possible to establish the spectra of specificity of the different extracts.

The alcohols used include molecules of C_1, C_2, C_3, C_4, C_5 and C_8. They are, respectively, methyl, ethyl, *n*-propyl and isopropyl, *n*-butyl, *sec*-butyl and isobutyl, *n*-amyl and *n*-octyl alcohols; furthermore, ethylene glycol and glycerol have also served as a substrate. The conditions of determination are similar to those described for the determination of alcohol dehydrogenase with alcohol concentrations identical to those of the ethanol concentration. /324

The results are shown as a graph where the activities are expressed in a percentage of activity for ethanol taken as 100% (see Fig. 4).

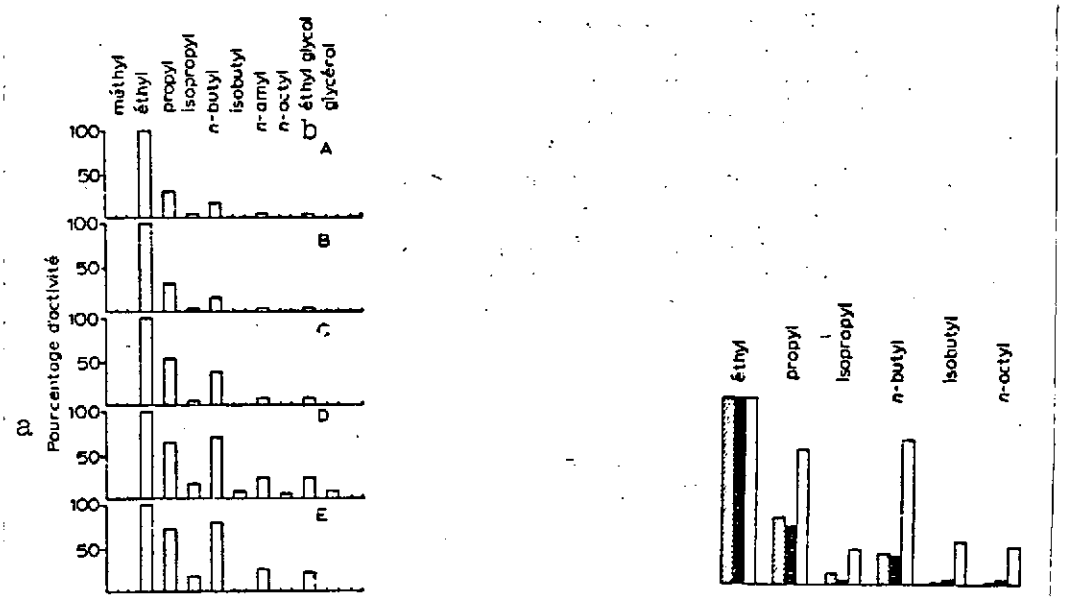

Fig. 4. Spectra of specificity of alcohol dehydrogenase of the different yeast extracts. A, glucose/air; B, glucose/nitrogen; C, glucose/air - end of growth; D, ethanol, and E, lactate.

Fig. 5. Spectra of specificity of alcohol dehydrogenase I (■), of alcohol dehydrogenase II (□) and of commercial crystalline alcohol dehydrogenase (▨).

Key: a. Percentage of activity
b. Ethylene glycol

The activities of alcohol dehydrogenase from S. cerevisiae extracts exhibit significant differences in specificity with respect to the alcohols studied, depending on the nature of the culture carbon substrate: while the alcohol dehydrogenase activities of yeast extracts cultivated on glucose, both in the presence and absence of oxygen, present great similarities, they are clearly distinguished from the yeast extracts from cultures on ethanol or lactate as the sole source of carbon, the latter activities behave in a similar manner one with respect to the other.

With respect to the change of carbon source that takes place naturally at the end of aerobic growth on glucose, it is accompanied by a change in the specificity of the extract opposite the alcohols, which reflects a variation of its composition in alcohol dehydrogenases.

Figure 5 includes, in one same graph, the alcohol dehydrogenase activities of commercial Boehringer crystalline yeast (values given by Barron and Levine [1]) and the relative activities of two soluble preparations of S. cerevisiae cultivated on glucose on the one hand (alcohol dehydrogenase I) and on ethanol on the other (alcohol dehydrogenase II).

The activities of alcohol dehydrogenase I are comparable to those of crystalline alcohol dehydrogenase, while the activities of alcohol dehydrogenase II are distinguished by a greater oxidation of the higher alcohols.

Oxidation of Cinnamic Alcohol

Ebisuzaki and Barron [2] described the presence of a second alcohol dehydrogenase in yeast, which they also called alcohol dehydrogenase II. Cinnamic alcohol, which is oxidized ten times faster by alcohol dehydrogenase II than by the classical

alcohol dehydrogenase appears among the alcohols that are oxidized by this new enzyme.

The study of the enzymatic specificity was extended to cinnamic alcohol in order to establish an eventual identity between the alcohol dehydrogenase II of Ebisuzaki and Barron and the heat-resistant alcohol dehydrogenase II revealed in the different yeast extracts during the present study.

Table V groups the relative activities for cinnamic alcohol of the different extracts, bearing in mind that a value of 100 is attributed to the activity opposite ethanol. /325

TABLE V. OXIDATION OF CINNAMIC ALCOHOL BY DIFFERENT EXTRACTS.

Origin of the Extract	Oxidation Activity of Cinnamic Alcohol
Glucose/N_2	4
Glucose/air (exponential phase)	4
Glucose/air (stationary phase)	26
Ethanol	36
Lactate	30

These results, which are close to those of Ebisuzaki and Barron, would appear to indicate that there is identity between the two enzymes, at least on the basis of the activity with respect to cinnamic alcohol.

Thermal Denaturation and Enzymatic Specificity

When the soluble fraction of a yeast extract grown on glucose is exposed to a heat treatment at 61° for 15 min, it loses 90% of its alcohol dehydrogenase activity. This moderate heat treatment produces the selective inactivation of the more thermosensitive enzyme, alcohol dehydrogenase I, without simultaneously denaturing the other component, alcohol dehydrogenase II (according to Fig. 3a).

Immediately following this treatment, the samples are cooled on ice and the oxidation activities of the different alcohols are then measured at room temperature.

Table VI shows the specificity results prior to and after the heat treatment.

TABLE VI. ENZYMATIC SPECIFICITY PRIOR TO AND AFTER A HEAT TREATMENT AT 61° FOR 15 MIN.

Alcohols	Prior to	After
n-Propanol	31	49
n-Butanol	15	36
Isobutanol	1.6	3.9
Cinnamic alcohol	6.5	21
Octanol	1.4	9.2

The specificity of the residual activity is substantially modified in the direction of an increased oxidation of the higher alcohols. Conversely, a similar heat treatment carried out with a /326 soluble extract of yeasts grown on ethanol does not modify the specificity.

Differential Synthesis Velocities of Alcohol Dehydrogenases During Prolonged Growth on Glucose

The resistance of alcohol dehydrogenase II to inactivation at the time of a moderate heat treatment has permitted the evaluation of its relative concentration in yeast extracts recovered at different optical densities.

The samples of soluble extracts are placed for 15 min at 61°, then cooled rapidly on ice. Their residual activity is then measured at room temperature.

The activities prior to and after this treatment, as well as the percentage of alcohol dehydrogenase II samples are set forth in Table VII.

TABLE VII. ACTIVITY OF THE TWO ALCOHOL DEHYDROGENASES THROUGHOUT PROLONGED GROWTH OF SACCHAROMYCES CEREVISIAE ON GLUCOSE.

Degree of Absorption	Activity of Alcohol Dehydrogenase (units)			% in Alcohol Dehydrogenase II
	Initial	After heat denaturation (alcohol dehydrogenase II)	Alcohol dehydrogenase I calculated by difference	
0.400	72.7	2.4	70.3	3.3
0.840	68.6	2.45	66	3.6
1.200	57.4	2.9	54.5	5.1
1.560	89.3	5.5	83.7	6.2
1.700	90.5	8.4	82	9.3
1.860	100.5	17.7	83	17.6
1.940	119.6	23.4	96	19.5
2.290	156	50	106	32
3.200	170	61	109	36

A graphic representation of the differential synthesis velocity of the different alcohol dehydrogenases during growth of S. cerevisiae on glucose is obtained measuring the quantity of synthesized dehydrogenase with respect to the quantity of proteins synthesized (Fig. 6).

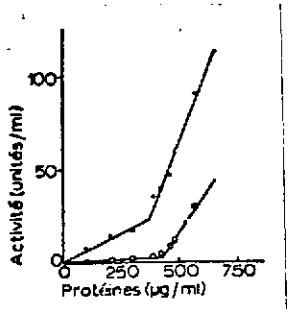

Fig. 6. Differential synthesis velocity of alcohol dehydrogenases. ●, total alcohol dehydrogenase; o, alcohol dehydrogenase II.

Key: a. Activity (units/ml)
b. Proteins

A clear discontinuity appears in the synthesis of the overall alcohol dehydrogenase, which is mainly the reflection of the start of the synthesis of alcohol dehydrogenase II. /327

Finally, these results permit the calculation of the differential synthesis

velocities of the different alcohol dehydrogenases in units of activity per 100 µg of proteins, and the evaluation of the rate of repression of the different enzymes during the exponential phase - stationary phase transition (Table VIII).

TABLE VIII. DIFFERENTIAL SYNTHESIS VELOCITIES AND DEREPRESSION RATES OF ALCOHOL DEHYDROGENASES.

Enzymes	Phases	Differential Synthesis Velocities (units/100 µg)	Rates of Derepression
Total alcohol dehydrogenase	Exponential	6	5.4
	Stationary	32.5	
Alcohol dehydrogenase I	Exponential	5.7	2.2
	Stationary	12.5	
Alcohol dehydrogenase II	Exponential	0.3	66.0
	Stationary	20	

Discussion and Conclusions

The comparative study of alcohol dehydrogenase activities present in S. cerevisiae grown on different carbon substrates proves the existence of two different proteins with alcohol dehydrogenase activity: alcohol dehydrogenase I, present mainly in yeast both in aerobic and anaerobic growth on glucose, and alcohol dehydrogenase II, responsible for the major part of the alcohol dehydrogenase activity of yeast extracts on ethanol. Insofar as the alcohol dehydrogenase activity of yeast extract on lactate is concerned, it is due to the presence of a mixture of the two types of enzymes in the extract.

It seems that the two enzymes can produce two distinct metabolisms, since they develop differently in the yeast, depending on the nature of the culture medium. Thus, alcohol dehydrogenase I, produced mainly by the yeast that assimilates the sugar according

to an essentially fermentative metabolism, must function in vivo in a physiological manner, namely, the reduction of acetaldehyde in ethanol. It is the fermentative alcohol dehydrogenase, whose specificity is marked by the acetaldehyde, which probably constitutes its only physiological substrate. On the other hand, alcohol dehydrogenase II, which develops when the yeast grows on ethanol as the only carbon source, belongs to the oxidative metabolism acting principally by oxidation of ethanol in acetaldehyde. It is the oxidative alcohol dehydrogenase, which has a high degree of specificity for C_3 and C_4 alcohols and which might also play a role in the detoxification of the cell opposite the higher alcohols.

The evolution of the yeast-cell content in the two alcohol dehydrogenases during aerobic growth on glucose confirms the role attributed to the two enzymes.

With the exception of the above-cited study of Ebisuzaki and Barron, few works led their authors to consider the possibility of a heterogeneity of the alcohol dehydrogenase of the yeast.

In a recent review, Sund and Theorell [9] estimate that the present results do not make it possible to definitely conclude that there are two different alcohol dehydrogenases in yeasts.

By means of the application of criteria such as thermostability and specificity, this work provides additional arguments in favor of the heterogeneity of alcohol dehydrogenase in yeast. Furthermore, the comparative study of the oxidation velocity of cinnamic alcohol by the two enzymes established a similarity between alcohol dehydrogenase II described by Ebisuzaki and Barron and the oxidative alcohol dehydrogenase, which would appear to definitely confirm the existence of the two different enzymes in S. cerevisiae.

The presence of two alcohol dehydrogenases in yeast thus constitutes a new example of the division of an enzyme involved in two metabolic pathways having a common stage to ensure coordination [6]. /328

Insofar as the regulation of alcohol dehydrogenases is concerned, the experiments described suggest that the alcohol dehydrogenase II synthesis is repressed by glucose. Actually, this enzyme appears either at the end of growth on glucose, or growing on a substrate such as lactate or ethanol. The facts observed by Galzy and Slonimski [3] thus correspond to the derepression of the oxidative alcohol dehydrogenase caused by the absence of glucose. With respect to the repression of alcohol dehydrogenase by glucose described by Witt, Kronau and Holzer [11], the evaluation of the derepression rates suggests that it is the oxidative alcohol dehydrogenase that is mainly repressed at the time of the growth of yeast on glucose. Conversely, the exhaustion of glucose in the medium modifies the rate of synthesis of the fermentative enzyme, whose regulation seems to be related to the constitutive enzyme, only slightly.

In conclusion, the S. cerevisiae yeast synthesizes two different alcohol dehydrogenases, both as a result of their thermostability, and of their specificity. Their physical preparation and their purification are currently being studied. This division of the enzymes, which makes it possible to independently regulate the two functions in which the alcohol dehydrogenase participates, raises the problem of the origin and evolution of these dehydrogenases within the framework of the chain of evolutive differentiation, which has led to the extremely large number of types in the yeast family.

Summary

Saccharomyces cerevisiae is able to synthesize two different alcohol dehydrogenases according to the culture carbon source: an

alcohol dehydrogenase I produced by the cell in aerobic or anaerobic growth on glucose (fermentative alcohol dehydrogenase) and an alcohol dehydrogenase II produced by the yeast grown on a respiratory substrate such as lactate or ethanol (oxidative alcohol dehydrogenase).

The difference between the two alcohol dehydrogenases was shown by the study of: (1) their heat denaturation; (2) their substrate specificity.

These two enzymes have common properties: both of them require NAD as a cofactor and both have the same optimal pH.

Alcohol dehydrogenase II is repressed by glucose.

Appreciation

We express our appreciation to Mr. J.M. Wiame for the interest he constantly expressed in this work.

We also express our appreciation to the Institut pour l'Encouragement de la Recherche Scientifique dans l'Industrie et l'Agriculture (IRSIA) who provided us with the means to do this work.

REFERENCES

1. Barron, E.S.G. and S. Levine, Arch. Biochem. Biophys. 41, 175 (1952).

2. Ebisuzaki, K. and E.S.G. Barron, Arch. Biochem. Biophys. 69, 555 (1957).

3. Galzy, P. and P.P. Slonimski, Compt. Rend. 245, 2556 (1957). /329

4. Hommes, F.A., Arch. Biochem. Biophys. 114, 231 (1966).

5. Kaplan, N.O., in Bryson, V. and H.J. Vogel, Evolving genes and proteins, Academic Press, New York, 1965, p. 243.

6. Kornberg, H.L., 15th Symp. Soc. Gen. Microbiol., University Press, Cambridge, 1965, p. 8.

7. Racker, E., Methods in enzymology 1, 500 (1955).

8. Schimpfessel, L., Doctoral thesis, 1964.

9. Sund, H. and H. Theorell, The enzymes 7, 25 (1963).

10. Wiame, J.M., Régulations chez les Micro-organismes [Micro-organism regulations], Coll. Intern. C.N.R.S., No. 124, 1965, p. 381.

11. Witt, I., R. Kronau, and H. Holzer, Biochim. Biophys. Acta 118, 522 (1966).

12. Yura, T., Proc. Natl. Acad. Sci. U.S. 45, 197 (1959).